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# Disturbance and resource availability act differently on the same suite of plant traits: revisiting assembly hypotheses

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**Abstract.** Understanding the mechanisms of trait selection at the scale of plant communities is a crucial step toward predicting community assembly. Although it is commonly assumed that disturbance and resource availability constrain separate suites of traits, representing the regenerative and established phases, respectively, a quantification and test of this accepted hypothesis is still lacking due to limitations of traditional statistical techniques. In this paper we quantify, using structural equation modeling (SEM), the relative contributions of disturbance and resource availability to the selection of suites of traits at the community scale. Our model specifies and reflects previously obtained ecological insights, taking disturbance and nutrient availability as central drivers affecting leaf, allometric, seed, and phenology traits in 156 (semi-) natural plant communities throughout The Netherlands. The common hypothesis positing that disturbance and resource availability each affect a set of mutually independent traits was not consistent with the data. Instead, our final model shows that most traits are strongly affected by both drivers. In addition, trait–trait constraints are more important in community assembly than environmental drivers in half of the cases. Both aspects of trait selection are crucial for correctly predicting ecosystem processes and community assembly, and they provide new insights into hitherto underappreciated ecological interactions.

**Key words:** canopy height; community assembly; disturbance; germination onset; leaf economic traits; nutrient availability; relative growth rate; seed mass; structural equation modeling; The Netherlands.

## INTRODUCTION

Understanding the causes of trait-based sorting among species during the assembly of plant communities is a crucial step toward predictive community ecology. Of particular interest is the role of environmental drivers in the filtering of traits during species sorting (*sensu* Keddy 1992). Several studies (Grime 1977, Tilman 1988, Lavorel and Garnier 2002, Westoby et al. 2002) have concluded that two drivers, resource availability and disturbance, are the most important in shaping species assemblages by selecting species having specific trait values that allow species to establish viable populations under particular levels of disturbance, resource availability (Lavorel et al. 1997, Osem et al. 2004, Fynn et al. 2005, Gross et al. 2007).

The effects of disturbance and resources on trait selection have been implicitly or explicitly hypothesized to generate different suites of traits (Lavorel and Garnier 2002, Grime 2006). For instance, disturbance, by promoting a variety of regenerative mechanisms, might predominantly act on traits determining regeneration, dispersal, and phenology (Grime 1977, 2006,

Grubb 1977, Lavorel and Garnier 2002). Resource availability would predominantly affect the established phase (*sensu* Grime 2006), acting on allometric and leaf traits (Aerts and Chapin 2000, Lavorel and Garnier 2002, Grime 2006). Additionally, several studies report a lack of correlation between traits of the regenerative and established phases (Shipley et al. 1989, Thompson et al. 1996, Grime et al. 1997). Given this, one generally expects correlations involving traits within these two types, but not involving traits between the regenerative and established phases unless the underlying drivers (disturbance and resource availability) are also correlated. Following the hypothesis of a mutually independent set of traits, Lavorel and Garnier (2002) argue that disturbance will not affect biochemical cycling, because regenerative traits are unconnected to these cycles. In contrast, nutrient availability would primarily affect biochemical cycling via modulation of traits of the established phase.

There are arguments against the assumed independence of these two types of traits. Since frequent and intense disturbances select for early reproduction and a greater allocation of resources into reproduction instead of growth, this could indirectly select for a short mature stature and against allometric traits that increase life expectancy, thus generating correlations between traits

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of the regenerative and established phase. Similarly, both high levels of disturbance and high levels of resource availability are expected to select for rapid growth rates. Although these arguments make sense intuitively, no study has tested the hypothesis that resource availability and disturbance select for separate suites of functional traits (as opposed to simply testing for an independence between these suites).

Some studies have determined the combined effect of disturbance and resource availability on plant traits (Fernandez et al. 1993, Fynn et al. 2005, Kuhner and Kleyer 2008), but they involved a limited range of habitats, mainly grasslands, and were not able to separate the effect of disturbance and resource availability. Another set of studies has established experimentally controlled gradients of nutrient availability and disturbance frequency or intensity (Grime 1977, Tilman 1988, Campbell and Grime 1992, Turkington et al. 1993), and then seeded out multispecies assemblages in order to investigate how these drivers differentially affect community structure and dynamics. Although these studies did not explicitly measure changes in the relative abundance of trait values, this would be possible in principle. The advantage of such studies is that the underlying environmental drivers are experimentally imposed, making causal inferences simpler. However, such experiments are necessarily of short duration relative to typical successional dynamics, involve relatively few species, and exist at very small spatial scales; longer-term and larger-scale data are also needed in order to increase generality and realism.

Although such data are necessarily observational in nature, specific multivariate causal relations between individual traits and environmental drivers, like those hypothesized in literature, can be made explicit in a structural equation model (SEM). In contrast to classical statistical techniques, a SEM is amenable to hypothesis testing and falsification (Shipley 2002) and quantifies the (relative) strength of hypothesized cause-effect relationships. In addition, the extent to which traits constrain other traits can be quantified once a well-fitting model is obtained. This is essential when determining the relative importance of environmental drivers in the selection of traits at the community level. In this study we quantify the effect of disturbance and nutrient availability on the selection of traits at the community level relative to each other and to trait-trait constraints across a wide range of habitats. Water availability is excluded because water is the least limiting resource in The Netherlands (mean annual precipitation is 754 mm, and the precipitation surplus is 191 mm) (Royal Netherlands Meteorological Institute, *available online*),<sup>5</sup> and its incorporation would unnecessarily increase the complexity of the model (see Appendix A for justification).

Although we have argued against the commonly accepted paradigm of independence of two suites of traits (as, e.g., proposed by Lavorel and Garnier [2002] and Grime [2006]), we start with formally testing it by SEM. Upon falsification of the model, we will proceed using SEM in a more exploratory fashion (Grace 2006), and will aim to (1) quantify the extent to which traits are affected by disturbance and nutrient availability, (2) quantify the magnitude of the direct and indirect effects (relationships of a driver that is transmitted via some intermediate trait) of a driver on each trait, and (3) identify and quantify the relative importance of trait-trait constraints independent of these environmental drivers.

## METHODS

### *Data acquisition and data selection*

*Plot selection.*—A database was compiled that contained information about species composition, nutrient availability, and disturbance in 156 plots from natural ecosystems throughout The Netherlands, a country with a temperate climate and small spatial differences in meteorological conditions. Six data sources were selected: Ertsen et al. (1998; 57 plots), Kemmers et al. (2001; 12 plots), van Dobben and de Vries (2001; 32 plots), Olde Venterink et al. (2002; 28 plots), Stuijzand et al. (2005; 5 plots), and Ordoñez et al. 2010b; 22 plots) (see Appendix B: Table B1 for detailed information). Together, this database covers a range from dry to wet, nutrient-poor to rich, and frequently disturbed to undisturbed habitats. They represent the major vegetation types in The Netherlands: dry and wet heath, dunes, grasslands, shrublands, and forests. Very frequently disturbed, nutrient-rich plots were not included in the data set, as these are heavily affected by agricultural use (crops and pesticides). The size of the sampled plots followed the standards of vegetation science of increasing sampling area with the size of plants in the plot (Schaminée et al. 1995). Plot sizes ranged from  $\sim 4$  m<sup>2</sup> in grasslands to  $\sim 100$  m<sup>2</sup> in forests. Species absence/presence data were available for each plot.

*Estimates of disturbance and nutrient availability.*—Data on total soil carbon, nitrogen, and phosphorus measurements were available for the 156 plots. From this we calculated the log<sub>10</sub> carbon-to-nitrogen ratio (soil C/N ratio) and log<sub>10</sub> carbon-to-phosphorus ratio (soil C/P ratio) as proxies for nutrient inavailability and quality of the soil organic matter. The soil pools were determined in soil samples taken in each ecosystem plot to a maximum of 20 cm depth. The number of replicates differed per study. For studies that measured organic matter content instead of total carbon, a conversion factor was applied, assuming that 48% of organic matter is made up of carbon. Total nitrogen and phosphorus were obtained from Kjeldahl, HCl, and H<sub>2</sub>O<sub>2</sub> destructions, assumed to give comparable estimates. In addition, total nitrogen was obtained from elemental analyzer determinations.

<sup>5</sup> www.knmi.nl

TABLE 1. Traits and the number of species involved in analyses of a database of plant species composition, nutrient availability, and disturbance in 156 plots from natural ecosystems in The Netherlands.

Trait category	Trait (acronym)	Scale and units	No. species
Leaf traits	leaf nitrogen content (LNC)	continuous (mg/g)	176
	leaf phosphorus content (LPC)	continuous (mg/g)	158
	specific leaf area (SLA)	continuous (mm <sup>2</sup> /mg)	267
Allometric traits	maximum canopy height (maxCH)	log <sub>10</sub> (continuous) (m)	285
Relative growth rate	relative growth rate (RGR)	continuous (g·g <sup>-1</sup> ·d <sup>-1</sup> )	113
Seed traits	seed mass of the germinule (SM_g)	log <sub>10</sub> (continuous) (mg)	262
Phenology traits	germination onset (GO)	ordinal: 0, germination in shoulder seasons (September–May); 1, no preference; 2, germination in summer (June–August)	269

Notes: Overall, 346 species were present in the plots. For all traits except phenology, the source was Douma et al. (*in press*). For phenology traits, the source was BioBase (2003), Centraal Bureau voor de Statistiek, Voorburg/Heerlen, The Netherlands.

Disturbance can be divided into the frequency and the intensity of disturbance. Following Grime and others, we defined disturbance as “any biomass removal leading to partial or total destruction of living biomass” (Grime 2001:80). This definition closely reflects the effect of disturbance on plant communities and enables comparison of the effect of different disturbance agents on one common scale (biomass removal). Unfortunately, we do not have any measure of the intensity of disturbance (relative amount of biomass removal compared to its yearly productivity). Therefore, our only measure of disturbance frequency was the time since the last major disturbance (years; cf. Falster and Westoby [2005] and Vile et al. [2006]).

**Trait selection.**—A species-trait database was compiled for species in The Netherlands, taken from Douma et al. (*in press*) and BioBase (2003). We selected seven traits, together covering various aspects of plant functioning, and which can be reasonably assumed, based on literature, to be under selective pressure of the environmental filters under investigation. Traits assumed to be related to regenerative phase were: seedling relative growth rate (RGR in units of per day); seed mass of the germinule (SM\_g in milligrams; seed without fruits or detachable appendages) and a phenology trait: germination onset (GO ordinal, 0, 1, 2: 0, germination in nongrowing seasons (September–May); 1, no preference; 2, germination in summer (June–August)). Traits assumed to be related to the established phase were three leaf traits, specific leaf area (SLA in square millimeters per milligram), leaf nitrogen content (LNC in milligrams per gram), leaf phosphorus content (LPC in milligrams per gram), and an allometric trait, maximum canopy height (maxCH in meters). The number of species for which trait data were available is shown in Table 1.

We coupled the species-trait database to the plot-species recording database to construct a plot–trait database, and selected those plots that contained trait information for the majority of the species (minimally 50%, but on average 85%; see Appendix C) for all traits, assuming that these species give a good estimate of the “real” plot trait mean. For LPC and RGR, this

minimum was lowered to >20% (leading to on average 69% and 48% species cover for LPC and RGR, respectively), as these traits are considered core traits; LPC is involved in energy storage and transfer and RGR reflects a plant’s growth potential (Lambers and Poorter 1992). Allowing missing trait data for calculating the plot mean did not significantly affect the trait–trait nor trait–environment patterns (see Appendix C for a detailed justification). In addition, the uncertainty in the slope estimates (as measured by an increase in the standard error of the slope) was found to be relatively robust to missing trait data (Appendix C). The restrictions caused by trait availability did not eliminate specific species assemblages from the data set, and therefore the sites selected were not biased compared to the total available sites. Overall, these restrictions led to the 156 plots previously described. The disturbance agents in the final data set were mowing (87), grazing (12), sod cutting (5), and trampling (3).

#### Data analysis

**Trait averages of species assemblages.**—Ackerly and Cornwell (2007) show that the average response of species to environmental drivers can be expressed by the plot mean values of the traits, because species filtering takes place at the community level. We considered community mean trait values based on both unweighted averaging and by weighting proportionate to species cover. There was no difference between the weighted and unweighted mean trait values in relation to nutrient availability and disturbance. This is in agreement with several other studies that report no qualitative difference between weighted and unweighted plot means (Ackerly and Cornwell 2007, Ordoñez et al. 2010b). However, the trait–trait correlations were stronger with the unweighted mean trait values. Therefore we used the unweighted plot mean values for the present study. Three traits were log<sub>10</sub>-transformed prior to analysis, two of them because their geometrical mean is more closely related to their ecosystem functioning, maxCH and SM\_g (Leps et al. 2006), and one (GO) was log<sub>10</sub>-transformed to ensure linearity with the environmental drivers (a requirement of SEM).

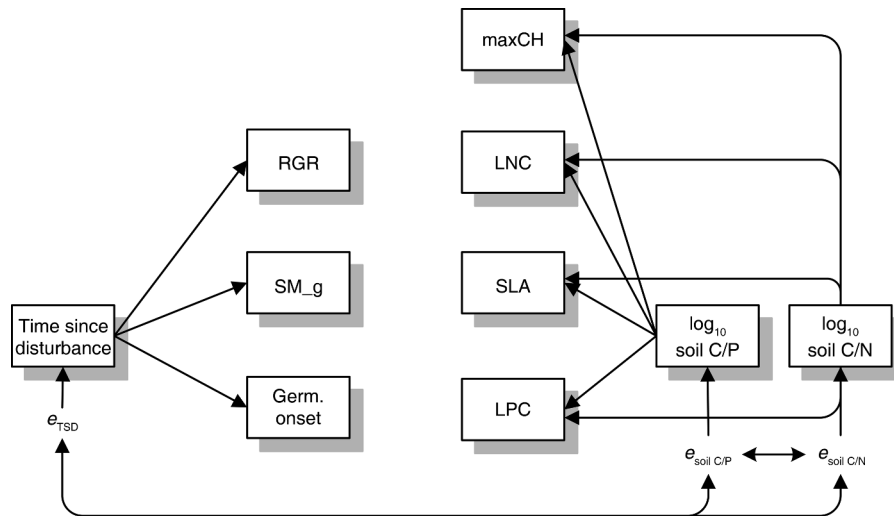


FIG. 1. Hypothesized structure of the effect of disturbance and nutrient availability on traits, assuming the current hypothesis that there are two sets of mutually independent traits, once any correlation between disturbance and nutrient availability is removed. See Table 1 for an explanation of trait abbreviations. Measured variables are represented by boxes. Causal relationships are represented by a single-headed arrow, and correlational relationships by a double-headed arrow between the error terms. For visualization, error terms representing all unexplained causes of a variable are not included. The model did not fit the data ( $\chi^2 = 702.74$ ,  $df = 32$ ,  $P < 0.0001$ ).

#### *Explaining multivariate patterns in traits among species assemblages*

The covariance among trait averages of species assemblages was analyzed first without explicitly defining *possible* underlying causes of common axes of variability between plots by submitting 156 plots  $\times$  7 traits to a principal component analysis (PCA; ter Braak 1987). Subsequently, we explicitly constrained the multivariate structure in traits by environmental data, but still without imposing any causal hypotheses, using a redundancy analysis (RDA; ter Braak 1987) based on three environmental variables (soil C/P ratio, soil C/N ratio, “time since disturbance”). These analyses provide insight into the multivariate pattern of traits (PCA) and how environmental drivers underlie this pattern (RDA).

#### *Explaining the covariance of traits by cause–effect relationships in a SEM*

As a third step, structural equation modeling (SEM) (for methodological details; see Shipley [2002] and Grace [2006]) was used to explicitly test hypothesized causal relationships. The model does not include feedbacks as all environmental variables were measured only once in time. We started with testing the commonly accepted paradigm of Lavorel and Garnier (2002) and Grime (2006) that disturbance and resource availability constrain separate suites of traits representing the regenerative and established phases, respectively. The causal structure posits that (1) traits related to regeneration, dispersal, and phenology respond only to disturbance and are correlated only because of their common selective response to disturbance; (2) leaf and allometric traits related to nutrient availability respond only to

selection based on this driver and are correlated only because of this common selective response (Fig. 1). As the proposed model appeared not to be consistent with the empirical data, we proceeded to modify it, going from a strict confirmatory analysis to an exploratory one. The modifications made to the original model, including support by literature, are described in a stepwise fashion in the *Results*.

The degree of fit of all hypothesized models, given the observed data, was measured using the Satorra-Bentler (Satorra and Bentler 1988) robust maximum likelihood chi-squared statistic, which corrects for nonnormality by comparing the predicted and observed covariance matrices. This statistic follows a chi-squared distribution with appropriate degrees of freedom if the data were truly generated according to the hypothesized causal structure. Significant lack of fit ( $P < 0.05$ ) means that the hypothesized model must be rejected as a causal explanation.

All individual cause–effect relationships were tested for significance with  $z$  statistics ( $H_0$ : path coefficient is zero). We use standardized path coefficients in order to compare effects by different variables in common units by dividing each measured variable by their standard deviation (Shipley 2002). A standardized path coefficient measures the degree to which one variable affects another, while controlling for other (causally prior) variables in the model. In this way it is possible to determine the relative importance of disturbance, nutrient availability, and trait–trait constraints (i.e., relationships between traits not mediated by the environmental drivers) on trait selection. The direct effect of an environmental driver (time since disturbance



or soil C/P and soil C/N) on a trait is the standardized path coefficient between it and the trait. The partial indirect effect of a driver on a trait along a given path is the product of the path coefficients along this path. The total indirect effect of a driver on a trait is the sum of the partial indirect effects along all paths going from the driver to the trait. The total effect of a driver on a trait is equal to the sum of all total direct and indirect standardized effects affecting that trait. The proportion of explained variance for all individual traits was calculated for all models to determine to what extent the most important cause–effect structures had been incorporated in the models.

The PCA and RDA were performed in R (R Development Core Team 2009), package *vegan* (Oksanen et al. 2008). SEMs were done using EQS 6.1 for Windows (Bentler and Wu 2005, Bentler 2006).

## RESULTS

### *Explaining variation between species assemblages with multivariate analysis: the importance of nutrient availability and disturbance*

A large percentage (80%) of the functional variation in plot trait means was explained with two PCA axes (Fig. 2a). Traits that were related to each other are maximum canopy height, seed mass of the germinule, and germination onset. Traits orthogonal to the first set of traits were the leaf traits (leaf nitrogen content, leaf phosphorus content, specific leaf area). Relative growth rate took an intermediate position between the two groups of traits (see Appendix D: Table D1). These results are comparable to a PCA of 8988 plots covering a wide range of environmental conditions in The Netherlands (see Appendix D: Fig. D1 [Douma et al., *in press*]). Moreover, extending the list of traits to 10, by adding flowering onset, seed mass including the dispersule and growth form, yielded a highly similar PCA (Appendix F), with allometric traits, seed traits, and phenology traits associated with the first PCA axis and leaf trait with the second PCA axis.

Environmental drivers explained up to 37% of the total variation in plot mean traits in a RDA, which was about half of the potentially explained variance (80%). Time since disturbance was most strongly related to the first RDA axis, while soil C/P ratios and soil C/N ratios were most related to the second axis, indicating the importance of these environmental variables in determining trait variation between assemblages (see Appendix D: Table D2). Soil C/P ratio was more strongly related to the second axis than soil C/N ratio.

### *Explaining the covariance of traits by cause–effect relationships*

We first tested the hypothesis that disturbance and nutrient availability each affect a different suite of traits, disturbance affecting the regenerative traits and nutrient availability the traits of the established phase. For this purpose the traits introduced before were classified into

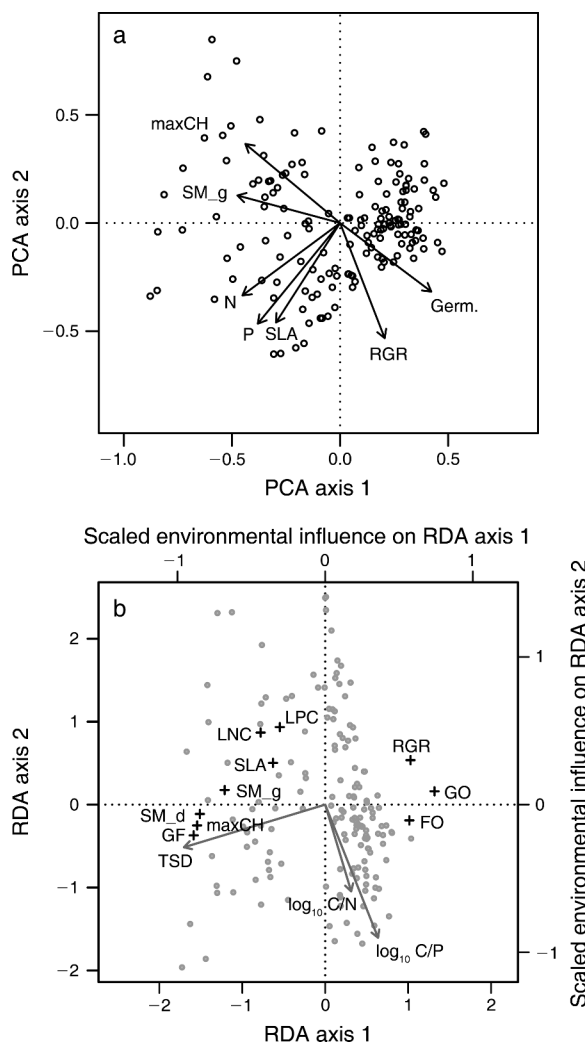


FIG. 2. (a) Principal component analysis (PCA) of the plot–trait matrix (156 relevés  $\times$  7 traits) from a database of plant species composition, nutrient availability, and disturbance in 156 plots from natural ecosystems in The Netherlands. For each plot, a trait mean was calculated for all seven traits (for details see *Methods: Data acquisition and data selection*). The first two axes explained 80% of the variation. (b) Redundancy analysis (RDA) with the plot–traits (156 plots  $\times$  7 traits) constrained by three environmental factors (time since disturbance (TSD),  $\log_{10}$  soil C/N ratio, and  $\log_{10}$  soil C/P ratio) explaining 38% of the total variation. Abbreviations of traits: LNC, leaf nitrogen content; LPC, leaf phosphorus content; SLA, specific leaf area; SM\_g,  $\log_{10}$  seed mass of the germinule; maxCH,  $\log_{10}$  maximum canopy height; RGR, seedling relative growth rate; GO,  $\log_{10}$  germination onset. The top and right-hand axes, respectively, show the scaled influence of the environmental drivers on RDA axis 1 and RDA axis 2.

two categories: seed mass, germination onset, and RGR were classified into the regenerative traits, while the leaf traits and maxCH were classified into the traits of the established phase. This model (Fig. 1) was not consistent with the data and was strongly rejected ( $\chi^2 = 702.74$ ,  $df = 32$ ,  $P < 0.0001$ ; a covariance matrix of the variables is presented in Appendix E: Table E1). After controlling

for any correlation between levels of disturbance and nutrient availability, there are not two sets of mutually independent traits, each associated with one driver only. We therefore proceeded with testing a series of models to deal with aims 2 and 3 posed in the *Introduction*, and to propose a new composite model. This new composite model is a modification of the confirmatory figure, but includes the joint effect of environmental drivers (step 1) on traits and trait–trait constraints (step 2). Since there is not much theoretical or empirical work that can be used to specify a priori causal hypotheses involving disturbance-related traits, phenology traits in particular, we proceeded in a more exploratory mode but with certain empirical and/or logical constraints derived from literature.

*Step 1: including the effects of nutrient availability and disturbance on traits.*—We started by adding paths from the environmental drivers to nonleaf traits for which relationships have been described in literature. A path from the two indicators of soil fertility (soil C/P and soil C/N) to RGR was added, since species from low-fertility environments have a lower growth rate than species from high-fertility environments (Lambers and Poorter 1992, Aerts and Chapin 2000). Additionally, a path was added from soil C/P and soil C/N to seed mass, as there are empirical studies reporting systematic variation between nutrient availability and this trait (Burke and Grime 1996, Ozinga et al. 2004, but see Kuhner and Kleyer 2008). Finally, germination onset was also made a function of soil fertility (Fenner and Thompson 2005).

Paths were added from “time since disturbance” to traits related to the established phase. We specified time since disturbance  $\rightarrow$  maxCH, as shifts in height are the most apparent change along a gradient of succession. Succession in habitats is mainly driven by the absence of disturbance (Chapin et al. 2002), because as soon as time since disturbance increases, height is not constrained anymore and the upward race for light starts (Westoby 1998). The effect of disturbance on the leaf traits is less clear. Diaz et al. (2001) reported that disturbance, via grazing, leads to a decrease in SLA. Therefore, a path was added from time since disturbance to SLA. For consistency only (as there has been no prior research on these relationships), paths were also added from time since disturbance to the other leaf traits: LNC and LPC. This model had a much lower  $\chi^2$  compared to the model proposed in Fig. 1, but was still not consistent with the data (Appendix E: Fig. E1,  $\chi^2=411.83$ ,  $df=21$ ,  $P < 0.0001$ ). We therefore proceeded with adding trait–trait constraints

*Step 2: including the effects of trait–trait constraints.*—The nonfitting model of step 1 suggests that relationships between traits are important on top of the constraining effect of the environmental drivers. Therefore we added a path from maxCH to SM\_g, because there is a well-established relationship between the two, very likely because “large species require a long juvenile period to become large individuals, and to survive a long juvenile period requires a high juvenile survivorship,

which is associated with large seeds” (Moles et al. 2004:394). Additionally, it is well established that there is an interspecific correlation between RGR and seed mass (Reich et al. 1998), although there is no good causal explanation for this pattern. We therefore added a free covariance between SM\_g and RGR. (In contrast to causal connections free covariances do not assume a causal direction between two variables; in the figures this is indicated with a double-headed arrow between the error terms.) RGR was additionally constrained by maxCH, as taller species (across growth forms) generally have a lower growth rate than smaller species (Cornelissen et al. 1996). MaxCH also affects leaf traits, in particular SLA, because smaller species tend to have a higher SLA than larger species to increase light capture (Falster and Westoby 2005). This was included by adding a path from maxCH to SLA. In addition, a path was added from maxCH to LNC.

Following the findings of Wright et al. (2004), the leaf traits were further constrained by a common cause, the leaf economics spectrum. This common cause is modeled as a “latent” variable, since exact measurements are not available (depicted in the figures as an oval). This latent variable is also included in the SEM of Ordoñez et al. (2010b), and is hypothesized in the SEM of Shipley et al. (2006) to be the ratio of cytoplasm volume and cell wall thickness. The scale of a latent can either be defined by fixing the path coefficient (the latent gets the same units as one of its indicators), or by setting the variance to 1. For the latent of the leaf economics, we fixed the variance of the latent to 1, treating the latent as a standardized variable and fixing its scale to standard deviation units (Shipley 2002). Testing this model resulted in a poor fit ( $\chi^2 = 144.82$ ,  $df = 12$ ,  $P < 0.0001$ , CFI (comparative fit index) = 0.86). Also the CFI showed that the model was not acceptable (CFI = 0.86, CFI ranges from 0–1,  $>0.95$  indicating an acceptable model, Shipley 2002)).

A model that was consistent with the empirical data was achieved by adding free covariances from the leaf economics latent to seed mass, relative growth rate, and germination onset. Testing this model resulted in a good fit ( $\chi^2 = 14.34$ ,  $df = 9$ ,  $P = 0.11$ , CFI = 1.00). Removing nine paths (that of TSD to LNC, LPC, GO, and SM\_g and soil C/N to GO and RGR and maxCH, soil C/P and SM\_g, GO) that were not significant, led to a model with an even higher fit ( $\chi^2 = 21.43$ ,  $df = 18$ ,  $P = 0.26$ , CFI = 1.00: Fig. 3). The explained variance of the traits by the hypothesized cause–effect relationships was high, in all cases exceeding 58% (Table 2). Parameter estimates, standard errors and the modeled covariance matrix are given in Appendix E: Table E2.

An alternative SEM modeled RGR in a different way; SLA and net assimilation rate (which approximates whole-plant net photosynthesis) are two of the three classical components of RGR (Lambers and Poorter 1992), and leaf photosynthesis is determined by leaf nitrogen levels (Wright et al. 2004). We therefore added

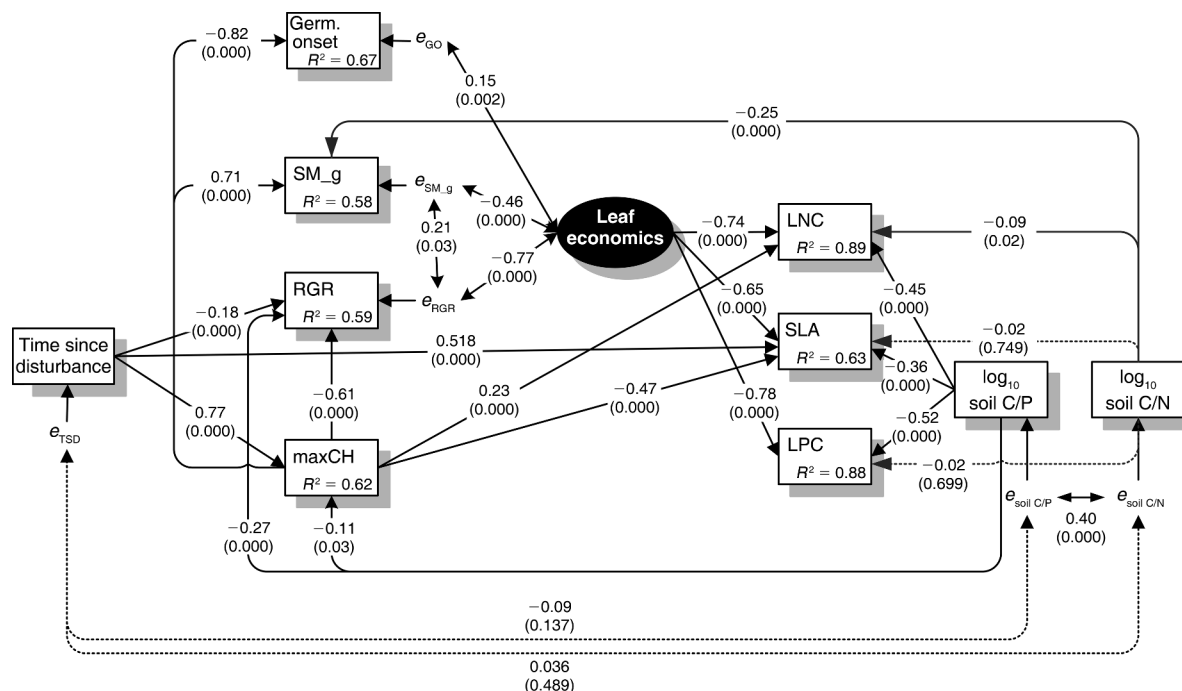


FIG. 3. Standardized path coefficients, explained variance (in boxes), and significance values (enclosed in parentheses) of the final model of nutrient availability, disturbance, and their related traits ( $\chi^2 = 21.43$ ,  $df = 18$ ,  $P = 0.26$ ). Dashed lines indicate nonsignificant relationships. The oval represents a latent variable (see *Methods: Step 2: including the effects of trait–trait constraints*). Measured variables are represented by boxes. Causal relationships are represented by a one-headed arrow. For visualization, error terms ( $e$ ) representing all unexplained causes of a variable are not included, except when used to indicate correlational relationships between variables (double-headed arrow between the error terms). For abbreviations of traits, see Fig. 1.

SLA→RGR and LNC→RGR to the previous model and removed the free covariance of RGR to the latent. This led to a model that was on the edge of significance ( $\chi^2 = 30.38$ ,  $df = 19$ ,  $P = 0.05$ , CFI = 0.99). A model with a higher fit was obtained ( $\chi^2 = 24.26$ ,  $df = 18$ ,  $P = 0.15$ ) by adding a free covariance between LNC and SM\_g, although we can't think of a common cause of both. For this reason we prefer the model described in the previous paragraph (Fig. 3).

In addition to the models presented before, a more complex model was developed, which in addition to the traits described before, included seed mass of the dispersule, flowering onset, and growth form. This extended model qualitatively confirms the patterns described above, showing the consistency and thus presumably ecological relevance of the paths independent of model configuration. We refer to Appendix F for details about this model configuration.

TABLE 2. The effect of environmental constraints (cause, columns) on the selection of individual traits (effect, rows) relative to the effect of trait–trait constraints.

Effect	Environmental constraint			Trait–trait constraints†				$R^2$ for final model
	Nutrient availability	Time since disturbance	Direct effects > indirect effects	Leaf traits	Allometric traits	Dominant driver	Dominant trait	
LNC	0.33	0.11	yes	<b>0.43</b>	0.14	nutrients	leaf traits	0.88
SLA	0.20	0.10	no	<b>0.41</b>	0.30	nutrients	leaf traits	0.63
LPC	0.40	0.00	yes	<b>0.60</b>	0.00	nutrients	leaf traits	0.88
RGR	0.14	<b>0.44</b>	yes	0.00	0.42	disturbance	allometry	0.59
maxCH	0.12	<b>0.88</b>	yes	0.00	0.00	disturbance	no data	0.62
SM_g	0.20	0.35	no	0.00	<b>0.45</b>	disturbance	allometry	0.58
GO	0.06	0.41	no	0.00	<b>0.53</b>	disturbance	allometry	0.67

*Notes:* The total effects of the two environmental drivers and the trait–trait constraints add to 1. The effect of the environmental drivers on traits is decomposed in both direct effects (DE) and indirect effects (IE, effects transmitted via other traits; Fig. 3). Trait–trait constraints were grouped into two categories: leaf traits (LNC, LPC, and SLA, leaf economy latent) and allometric traits (represented by maxCH). Additionally, the dominant environmental driver and the dominant trait–trait constraints, as well as the explained variance of the traits, are shown. For an explanation of the trait abbreviations, see Table 1.

† Note that the total effects do not (by definition) include free covariances between variables (see also *Discussion: An important role for trait–trait constraints in trait assembly*).



To answer the third research question, the relative effects of environmental drivers on traits were calculated using the fitted model in Fig. 3. These calculations showed that soil C/P and soil C/N predominantly constrained leaf traits, such as SLA, LNC, and LPC, and that time since disturbance predominantly affected maxCH, SM\_g, RGR, and GO (Table 2). However, the effect of both drivers was not simply restricted to one suite of traits, but affected both suites of traits simultaneously. For example SM\_g was almost equally affected by nutrient inavailability and time since disturbance (0.20 vs. 0.35). The constraining effects of the individual environmental drivers on traits were only in two out of seven traits stronger than trait–trait constraints. In three out of seven traits, the indirect effect of an environmental driver (i.e., mediated via a trait) was stronger than its direct effect. Calculating the relative effects of the environmental drivers on traits of the alternative model (effect of SLA and LNC on RGR) gave similar results, with the exception that the constraints of SLA and LNC (leaf traits) on RGR was stronger than those of plant allometry (via maxCH).

#### DISCUSSION

##### *Resource availability and disturbance are important drivers of trait assembly*

Our aim was to quantify the direct and indirect effects of disturbance and nutrient availability on trait selection during community assembly relative to trait–trait constraints. The RDA showed that disturbance and nutrient availability are both important determinants of plant traits across habitats, confirming previous work (Grime 1977, Tilman 1988, Westoby 1998, Lavorel and Garnier 2002). In addition, they show that the accepted explanation that correlations between regeneration traits are driven by disturbance, whereas correlations between leaf traits are driven by nutrient availability is a good first approximation (with the exception of maxCH).

##### *Falsification of the commonly accepted paradigm of independent suites of traits*

Although disturbance and resource availability are important drivers of trait assembly, the way disturbance and resource availability affect different traits is clearly more complicated than current theory proposes (Lavorel and Garnier 2002, Grime 2006). The initial causal structure, positing that disturbance and resource availability each affect a set of mutually independent traits (Fig. 1), was not consistent with the empirical data, and thus cannot be accepted as a causal explanation of the complex trait patterns in species assemblages found in this data set. This was also true when the time since disturbance and soil C/P and soil C/N were allowed to covary, meaning that trait correlations were not simply induced by landscape correlations between the two gradients. Our final model shows that a more nuanced explanation is required: traits from the established and regenerative phase are not exclusively linked to only one

driver, but in most cases to both. In addition, for some traits, trait–trait constraints are more important than the individual effect of an environmental driver. Particularly with respect to the interaction between traits, there appeared to be several unknowns. This phenomenon will be discussed later in the article.

##### *About the specification of the alternative model*

The connecting paths that were included in the final model were based on relationships described in the literature and led to a model that was consistent with the data ( $P = 0.26$ ) with the signs of all individual paths according to expectations. The strength and direction of effect of maxCH on other traits should be interpreted as a combined effect of a shift in growth form and canopy height. This explains the negative effect of maxCH on RGR (which is positive when corrected for growth form; see Appendix F). The contrasting response of SLA and LNC to maxCH may be understood by the fact that LNC does not differ with growth form, while SLA does. (SLA is on average higher in herbs and grasses than in shrubs and trees [Ordoñez et al. 2010a].) The latent that was used to represent the leaf economics spectrum showed almost equal weights to the leaf traits as the latent in the study of Ordoñez et al. (2010a) on the same traits. This strengthens our interpretation of the latent. Nonetheless, the generality of this latent may benefit from more work on what ties the leaf traits together (Shipley et al. 2006, Blonder et al. 2011) and how this drives other traits. As such our model is open for improvement and has to be tested on an independent data set to prove its generality (Grace 2006). This caveat is especially important because our analysis involves both confirmatory components and exploratory modifications based on biological expectations and statistical considerations of model fit. In addition, several alternative models were shown to be consistent with the data. These models particularly differed in the role of leaf economy latent and plant allometry on traits. Therefore one should be careful with interpreting the relative effects of the trait–trait constraints (see *An important role for trait–trait constraints in trait assembly*). Note also that missing trait data and measurement errors probably introduced some error in the estimates of the path coefficients. As a result, the significance tests of the paths may be biased toward Type I errors. However, this will mainly affect paths with small path coefficients, for which the relative effects on trait selection would have been small anyway. Further work, in which independently established measurement models are used, including multiple environmental indicators to estimate a latent variable, is an important next step, but this cannot be done with our data. The model with three additional traits (Appendix F: Fig. F1) showed an even more complex interaction of environmental drivers and traits, but with more uncertainties, while the estimates of the direct and indirect effects of environmental drivers and of trait–trait constraints were comparable. For this

reason we will continue the discussion with examples from the model presented in Fig. 3.

*The relative role of nutrient availability and disturbance in trait selection*

Seed mass, germination onset, and relative growth rate were generally constrained by both environmental drivers, while leaf traits were predominantly constrained by nutrient availability, and allometric traits by time since disturbance (Table 2). The model shows that some traits can be clearly associated with one driver, although this does not preclude them from being associated with the other as well, while other traits are constrained equally by both drivers. SM<sub>g</sub> and RGR, particularly, appeared to be almost equally driven by both filters. In addition, the SEM model shows that disturbance and nutrient availability both have a direct and an indirect effect on traits. The use of SEM allows us to decompose the total effects of a driver on a trait into direct and indirect effects. In three out of seven cases (and six out of 10 in the complex model; Appendix F), the indirect effects of an environmental driver appeared to be more important than its direct effect, showing the importance of evaluating trait selection within the context of other trait and environmental relations. Furthermore, the indirect effect can be opposite to the direct effect. This was the case for SLA, in which the effect of disturbance via height was twice as strong and in the opposite direction as the direct effect of time since disturbance.

The relative effect of soil C/P and soil C/N on the leaf traits is rather low compared to the effect of the leaf economy latent on these traits. Probably the role of nutrient availability is underestimated, since both soil C/N and soil C/P ratios are imperfect approximations of nutrient availability as experienced by plants. Attempts to quantify the error associated with these measurements failed, since we do not know the nutrient availability as experienced by plants (see for a detailed discussion Ordoñez et al. [2010b]) and secondly, since we did not have alternative indicators for nutrient availability. Soil C/N and soil C/P ratio could not be used as indicators of a single latent, since each of the estimates had a unique effect on traits (model not shown). Soil C/N appeared to significantly constrain LNC and SM<sub>g</sub>, while soil C/P had a significant effect on all leaf traits. Future work would therefore benefit from measuring multiple indices of nitrogen and phosphorus availability to derive better estimates of soil nutrient availability. These multiple indices in combination with a latent variable can potentially provide estimates of the errors associated with these measures and can then be used to better estimate the relative effect of nutrient availability on traits.

*An important role for trait–trait constraints in trait assembly*

Although the environmental drivers were important in accounting for the trait correlations, the SEM shows clearly that these are not sufficient. The  $\chi^2$  dropped by a

factor of 20 when including trait–trait constraints, showing their important role in trait selection. In four out of seven traits, trait–trait constraints appeared to be more important than the (direct + indirect) effects of disturbance plus nutrient availability. Note that it is possible that two traits are correlated because of common selection by an environmental driver unknown in this study.

The leaf economy latent was most important for internal coordination of the leaf traits, while RGR, SM<sub>g</sub>, and GO were predominantly constrained by plant allometry (represented by maxCH). We hypothesize that the increased investment in structural tissues unavoidably requires adjustments of other traits, as investment in structural biomass leads to changes in life span, brings costs for tissue maintenance, water transport, and belowground tissues (Westoby et al. 2002, Falster and Westoby 2005) and subsequently may change investments in and timing of reproductive biomass (Leishman et al. 1995, Moles et al. 2004, Bolmgren and Cowan 2008). In contrast, surprisingly little is known about how (and if) leaf economy drives other traits, and the SEM identifies this knowledge gap. For example, in the final model and the more complex model of Appendix F, the model fit significantly improved if a relationship between LNC and SM<sub>g</sub> was added, which is, to our knowledge, not reported in the literature. Similarly, it is unknown how the leaf economy latent drives RGR, SM<sub>g</sub>, and GO. For this reason these relationships were modeled as free covariances. A consequence of our lack of knowledge concerning how these traits are coordinated is that the relative role of leaf economy vs. plant allometry in trait selection is dependent on the causal specifications of the model. For example the complex model (Appendix F) showed a larger role for allometry traits compared to leaf traits. In the model of Fig. 3, the role of the leaf economy latent on SM<sub>g</sub> and RGR and GO could have been larger if these relationships would have been modeled by a causal connection. Despite the lack of knowledge on causality between traits, trait–trait constraints are a substantial determinant of trait assembly. Therefore, they should be explicitly taken into account if one aims to predict the functional composition of communities.

## CONCLUSIONS

Resource availability and disturbance are often asserted to be the two main drivers of trait selection at the scale of plant communities. This paper provides a quantification of this assertion using a large-scale data set and shows that resource availability and disturbance can indeed explain a large proportion of the trait selection among communities. However, this analysis also shows that some important adjustments to current theory are required. These adjustments are twofold. First, most traits are simultaneously affected by both environmental drivers. This implies that, contrary to current hypotheses, disturbance can change biogeochemical cycling by modulating traits of the established

phase. Second, some traits are more constrained by other traits than by environmental drivers, even when accounting for indirect effects of these drivers. Both aspects, so far unquantified, are shown here to be critical for correctly predicting functional trait assembly and ecosystem processes.

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## SUPPLEMENTAL MATERIAL

### Appendix A

Justification for the omission of moisture supply in the SEM (*Ecological Archives* E093-073-A1).

### Appendix B

Data sets used for this study (*Ecological Archives* E093-073-A2).

### Appendix C

Control analyses for robustness against missing trait values (*Ecological Archives* E093-073-A3).

### Appendix D

Results of the PCA, RDA with seven traits and three environmental variables (*Ecological Archives* E093-073-A4).

### Appendix E

(Modeled) Covariance matrix and mathematical model specification of the final model (*Ecological Archives* E093-073-A5).

### Appendix F

Results of the PCA, RDA, and RDA with latents of 10 traits. Explanation and specification of the extended SEM (*Ecological Archives* E093-073-A6).